DEVELOPING THE CALCIUM-DEPENDENT CONFORMATIONAL BEHAVIOR OF THE RTX PEPTIDE DOMAIN FOR NOVEL PROTEIN CAPTURE AND RECOVERY APPLICATIONS

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Key Words: Beta Roll Domain, Non-chromatographic Protein Purification, Affinity Purification, Protein Hydrogels

The β-roll domain is a unique, conformationally dynamic peptide secondary structure motif[1]. This peptide is expressed from the repeats-in-toxin (RTX) domains found in some secreted pathogenic proteins. The peptide is intrinsically disordered in the absence of calcium. In calcium rich environments, the peptide binds Ca++ ions and folds into a β-roll secondary structure that resembles a flattened corkscrew. It is composed of two parallel β-sheet faces with a conserved aspartic acid at each turn that is responsible for the Ca++ binding. We have extensively characterized this calcium-responsive RTX domain and evaluated its potential as a new bioseparations platform in both non-chromatographic and affinity chromatography applications, as well as a novel component for the development of advanced protein hydrogels.

We have developed a synthetic peptide, based on RTX domains, which undergoes calcium-responsive, reversible precipitation. This synthetic tag was appended to green fluorescent protein, β-lactamase and alcohol dehydrogenase. After protease cleavage of the precipitating tag, pure and active target proteins were obtained by cycling precipitation steps before and after cleavage. This work demonstrates a new stimulus-responsive precipitating tag that can be used for efficient bioseparations using gentler conditions than existing alternatives, enabling purification of recombinant proteins from microbial lysate in only a few minutes[1].

More recently, we have also shown that this β-roll peptide domain can serve as a new scaffold for engineering controllable biomolecular recognition. Mutant peptide libraries were selected against lysozyme via ribosome display and we were able to identify peptides with mid-nanomolar (63μM) dissociation constants. We showed that the mutant RTX peptides are capable of capturing the lysozyme target in affinity chromatography experiments in the presence of calcium and the bound target is easily eluted upon removal of the calcium ions, in a Catch and Release mechanism[2].

We have previously mutated the amino acids on the faces of the β-roll peptide domain to hydrophobic leucine side chains to enable calcium-induced self-assembly. This provides cross-links that enables calcium-dependent protein hydrogel formation[4,5]. We have introduced our lysozyme-binding β-roll peptide into this platform so that the designed proteins are a viscous liquid in the absence of calcium. Upon calcium addition, a robust hydrogel is formed that specifically binds the target protein, lysozyme[6]. Thus the engineering of the peptide domains has led to new biotechnology applications, where environmentally responsive protein hydrogels are capable of selective and reversible protein capture and immobilization.

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